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ORIGINAL ARTICLE

Fascia tissue engineering with human adipose-derived stem cells in a murine model: Implications for pelvic floor reconstruction

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Background/Purpose: Mesh-augmented vaginal surgery for treatment of pelvic organ prolapse (POP) does not meet patients' needs. This study aims to test the hypothesis that fascia tissue engineering using adipose-derived stem cells (ADSCs) might be a potential therapeutic strategy for reconstructing the pelvic floor.
Methods: Human ADSCs were isolated, differentiated, and characterized *in vitro*. Both ADSCs and fibroblastic-differentiated ADSCs were used to fabricate tissue-engineered fascia equivalents, which were then transplanted under the back skin of experimental nude mice.
Results: ADSCs prepared in our laboratory were characterized as a group of mesenchymal stem cells. *In vitro* fibroblastic differentiation of ADSCs showed significantly increased gene expression of cellular collagen type I and elastin ($p < 0.05$) concomitantly with morphological changes. By contrast, ADSCs cultured in control medium did not demonstrate these changes. Both of the engrafted fascia equivalents could be traced up to 12 weeks after transplantation in the subsequent animal study. Furthermore, the histological outcomes differed with a thin (111.0 ± 19.8 μm) lamellar connective tissue or a thick (414.3 ± 114.9 μm) adhesive fibrous tissue formation between the transplantation of ADSCs and fibroblastic-differentiated ADSCs, respectively. Nonetheless, the implantation of a scaffold without cell seeding (the control group) resulted in a thin (102.0 ± 17.1 μm) fibrotic band and tissue contracture.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Conclusion: Our results suggest the ADSC-seeded implant is better than the implant alone in enhancing tissue regeneration after transplantation. ADSCs with or without fibroblastic differentiation might have a potential but different role in fascia tissue engineering to repair POP in the future.

Introduction

The lifetime risk of undergoing surgery to repair pelvic organ prolapse (POP) is 11—19% in the general female population.[1,2] However, attempts to correct this problem still result in a high failure rate and a significant number of patients need re-operation.[3] In order to improve surgical outcomes, the use of synthetic meshes for the reinforcement of POP repair has become popular.[4] Unfortunately, evidence of efficacy for these procedures is not convincing, and rates of complications such as vaginal erosions, pain, infections, and shrinkage are unacceptably high.[5,6,7] Recently, stem cell-based tissue engineering and cellular therapy, which are underway in genitourinary tract reconstruction,[8,9,10] have been proposed for treatment of POP to avoid the complications associated with surgical meshes.[11,12]

During the past decade, different types of stem cells, especially bone marrow-derived mesenchymal stem cells (MSCs) and skeletal muscle-derived stem cells, have been used in preclinical and clinical studies for treating stress urinary incontinence.[9,10] However, little effort has been made on the treatment of POP using these cells. Ho et al found that muscle-derived stem cells seeded into biological grafts stimulated vaginal tissue repair in rats and suggested that this might be a promising approach for vaginal repair.[13] Previously, we successfully fabricated a fascia equivalent *in vitro* and *in vivo* using human vaginal fibroblasts from patients with POP.[14] However, these options may not be optimal for the eventual implementation in POP repair because these stem and/or progenitor cells are difficult to obtain in adequate quantity and quality.

By contrast, adipose-derived stem cells (ADSCs), which are abundant and easy to obtain from discarded adipose tissue, have been used in stem cell-based therapy across several different medical disciplines.[9,15] In addition, some studies have shown that ADSCs have equal or superior therapeutic potential compared to bone marrow-derived MSCs.[16,17] In order to explore the potential of using ADSCs as a cell source for POP repair, we conducted this preclinical study. In this study, we tried to (1) isolate, expand, and characterize human ADSCs *in vitro*; (2) induce fibroblastic differentiation of ADSCs *in vitro*; (3) use ADSCs and fibroblastic-differentiated ADSCs to fabricate tissue-engineered fascia equivalents *in vitro* and evaluate the histological outcomes after *in vivo* transplantation. A murine model for experiments of tissue regeneration was used in this study.[14,18,19]

Methods

Tissue biopsy and cell isolation

A piece of subcutaneous adipose tissue was excised from the abdomen of women who had undergone gynecological surgery after informed consent was given using an Institutional Review Board (IRB)-approved protocol at our institution (TCVGH IRB No. S07125). ADSCs were isolated following the methods described by Zuk et al.[20]

Cell culture, expansion, and senescence assay

Following cell plating, the ADSCs were maintained in a humidified incubator at 37°C/5% CO_2 in control medium (CM) consisting of Dulbecco's modified Eagle medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin solution (Gibco) at a density of 5×10^6 nucleated cells/10-cm tissue culture dish. The medium was changed twice weekly thereafter. Cells were passaged with 0.25% trypsin/0.1% EDTA upon reaching 80% confluence. The senescent cultured cells were detected by the Senescence beta-Galactosidase Staining Kit during cell expansion (Cell Signaling Technology Inc., Beverly, MA, USA) according to the manufacturer's protocol.

Characterization of ADSCs *in vitro*

In vitro characterization was done according to the minimal criteria set by The International Society for Cellular Therapy in 2006 for defining multipotent MSCs.[21] Additional cell surface antigens were evaluated according to methods used by Mitchell et al[22] and Varma et al.[23] ADSCs were confirmed by the typical cell morphology, adherence to plastics, specific cell surface antigen expression, and potential for tri-lineage (i.e., adipogenic, chondrogenic, and osteoblastic) differentiation. Homogeneous populations of cultured adherent cells at passages 3—5 were used in the following experiments. Each experiment was repeated three times.

Induction of fibroblastic differentiation of ADSCs *in vitro*

Fibroblastic differentiation was performed according to the protocol described by Lee et al.[24] In brief, ADSCs were treated with CM supplemented with 100 ng/mL of recombinant human connective tissue growth factor (rhCTGF; PROSPEC Protein Specialists, East Brunswick, NJ) and 50 μg/mL ascorbic acid, and the medium was changed twice a week. At the same time, cultured adherent cells incubated in CM were used as a negative control. Following fibroblastic differentiation, the mRNA of extracellular matrix (ECM) molecules including collagen type I, collagen type III, collagen type V, elastin, and tenascin-C were assayed weekly by reverse transcription polymerase chain reaction (RT-PCR) at baseline until week 4. Meanwhile, two master transcription factors, peroxisome proliferator-

activated receptor-γ (PPAR-γ) for adipogenesis and Runt-related transcription factor 2 (RUNX2) for osteogenesis, were evaluated, respectively. PCR amplification was performed using the primer sequences established by GenBank. RT-PCR products were analyzed with conventional agarose gel electrophoresis and ethidium bromide staining. Densitometry with image analysis software (Bio1D; Vilber Lourmat, Marne La Vallée, France) was used to determine and quantify the intensity of the bands.

Fabrication of tissue-engineered fascia equivalents *in vitro*

The fascia equivalents were prepared according to previously described methods.[14] In brief, a prepared collagen solution containing ADSCs or fibroblastic-differentiated ADSCs (3.5×10^7 cells/mL in 2.15 mL of $1 \times$ DMEM supplemented with 23.3% FBS in final collagen concentration of 1.1 mg/mL) was applied to the biodegradable poly-DL-lactico-glycolic acid (PLGA) mesh (Ethicon, Edinburgh, UK), 1 cm $\times$ 1 cm in size. The scaffolds seeded with ADSCs or fibroblastic-differentiated ADSCs (tissue-engineered fascia equivalents) were kept in a submerged culture for 24 hours in standard culture conditions before the animal study. The seeded cells were labeled in advance with a dialkyl-carbocyanine fluorescent solution (Vybrant DiI; Molecular Probes, Eugene, OR) according to the manufacturer's protocol. Cells were stained and tracked *in vitro* and *in vivo* using the same dye.

Graft transplantation and tissue procurement

Animal studies were performed according to the guidelines set by the Animal Research Committee at our institution. Three sets (9 mice in each set) of experimental nude mice were prepared for the transplantation. The tissue-engineered fascia equivalents were transplanted under the back skin of experimental female nude mice (8 weeks old) (two sets). Meanwhile, PLGA mesh mixed with collagen gel without cell seeding was used for implantation as the control (1 set). At 4 weeks, 8 weeks, and 12 weeks after transplantation, the animals were sacrificed and tissue samples were harvested for subsequent histological analysis.

Histological analysis

All specimens were fixed with 4% paraformaldehyde and embedded with paraffin (H&E, Masson's trichrome staining) or O.C.T. compound (Tissue-Tek; Sakura Finetek, Torrance, CA, USA). Tissue sections were cut for histological examination. All slides were photographed, and images were digitalized using a color digital camera and imaging software (Spot, version 2.1; Diagnostic Instruments, Sterling Heights, MI, USA). Localization of DiI-labeled cells was determined using a fluorescent microscope (Axiovert 200M; Carl Zeiss, Oberkochen, Germany) filtered for excitation/emission at 546/590 nm.

Statistical analysis

All data were presented as mean $\pm$ standard deviation (SD). The statistical analysis was performed using one-way analysis of variance (ANOVA) repeated measurement for multiple comparisons. The level of statistical significance was set at $p < 0.05$.

Results

Tissue harvest and cell yield

The mass of adipose tissue harvested, number of isolated nucleated cells and adherent cells, and yield of adherent cells per gram of tissue sample from six women is summarized in Table 1. The average mass of harvested tissue was 5.47 ± 1.68 grams. The average yield of adherent cells was $0.83 \pm 0.22 \times 10^5$ per gram of harvested tissue.

Growth kinetics of ADSCs

To examine the long-term growth kinetics of ADSCs, we measured cumulative population doublings with respect to passage numbers (Fig. 1A). ADSCs underwent an average of 1.8 population doublings before the first passage. An average of 2.0 population doublings was observed upon subsequent passages. A linear relationship between cumulative population doubling and passage number was observed (P10 $= 85 \pm 9.1$ days in culture). Cell senescence in cultures at passages 1, 5, and 9 are shown in Fig. 1B,C, respectively. Cell cultures at passage 1 exhibited no appreciable beta-galactosidase staining. A significant increase in beta-galactosidase staining was noted in later passages. The percentage of senescent cells remained below 5% throughout the nine passages.

In vitro characterization of ADSCs

ADSCs expanded easily *in vitro* and exhibited a homogeneous cell population of the typical spindle-shaped cell morphology with adherence to plastics after the third passage (Fig. 2A). As expected, ADSCs expressed the MSC-associated antigens (i.e., CD29, CD44, CD73, CD90, CD105, CD166) at varying degrees, but did not (or minimally) express the hematopoietic stem cell-associated antigens (i.e., CD14, CD19, CD34, CD45, CD117) or endothelial cell-associated antigens (i.e., CD31, von Willebrand factor) or human leukocyte antigen (HLA)-DR molecules (Fig. 2B). The tri-lineage (i.e., adipogenic, chondrogenic, and osteoblastic) differentiation potential of ADSCs was checked by cytochemical staining with Oil Red-O, Alcian Blue, and Alizarin Red S (Sigma-Aldrich Inc., St. Louis, MO). The mean number of Oil Red-O (adipogenic), Alcian Blue (chondrogenic), and Alizarin Red S (osteogenic)-positive staining cells measured in six donors was $71.8 \pm 6.7\%$, $70.2 \pm 14.7\%$, and $58.2 \pm 9.9\%$ of the total ADSC number, respectively (Fig. 2C). By contrast, undifferentiated ADSCs at the same passage number did not show positive labeling for tri-lineage differentiation.

Fibroblastic differentiation of ADSCs

The differentiation induced a significant increase in the gene expression of collagen type I and elastin in ADSCs by both qualitative and quantitative analysis via RT-PCR

Table 1 Summary of the amount of adipose tissue harvested, total nucleated and adherent cells isolated, and cell yield from six women who had undergone gynecological surgery.

Patient data					Adipose tissue			
No.	Age	Parity	Menopause	BMI (kg/m^2)	Mass of harvested tissue (g)	Total nucleated cells ($\times 10^6$)	Number of adherent cells ($\times 10^5$)	Yield adherent cells ($\times 10^5$)/g
1	20	0	N	25.8	8.14	10.50	6.87	0.84
2	52	2	N	22.6	5.93	1.33	4.89	0.82
3	46	1	N	25.1	6.13	8.96	2.85	0.46
4	60	2	Y	24.0	4.53	10.50	4.35	0.96
5	53	3	N	30.1	4.90	1.61	5.52	1.13
6	49	1	N	26.0	3.20	8.00	2.53	0.79
Average	47	1.5		25.6	5.47	6.82	4.50	0.83

(Fig. 3A,B). By 4 weeks, the expression of the mRNA levels of collagen type I and elastin content in ADSCs increased significantly by approximately 2.0 and 1.8 fold, respectively, in comparison with ADSCs at baseline ($p < 0.05$). There were no statistically significant changes in the mRNA expression of other ECM molecules including collagen type III, collagen type V, and tenascin-C. However, the ratios of collagen type I/III and collagen type I/III+V increased progressively from baseline to 2.04 and 2.38 fold, respectively, after 4 weeks of culture. Meanwhile, the gene expression of PPAR-γ was not induced and that of RUNX2 was stable (Supplementary Fig. 1). In addition to changes in genetic expression, cell morphology changed progressively from a slender shape to a plump shape with more cytoplasm and a more vesicular-like nucleus (Fig. 3C). By contrast, no significant changes of genetic or morphological expression were observed in the control group.

Tissue-engineered fascia equivalents and experimental animal model

Before transplantation, tissue-engineered fascia equivalents were observed microscopically. The previously DiI-labeled ADSCs or fibroblastic-differentiated ADSCs were identified under fluorescence microscopy. Both the cell density and distribution were similar between groups (Fig. 4A,B). The histology of the normal back skin in

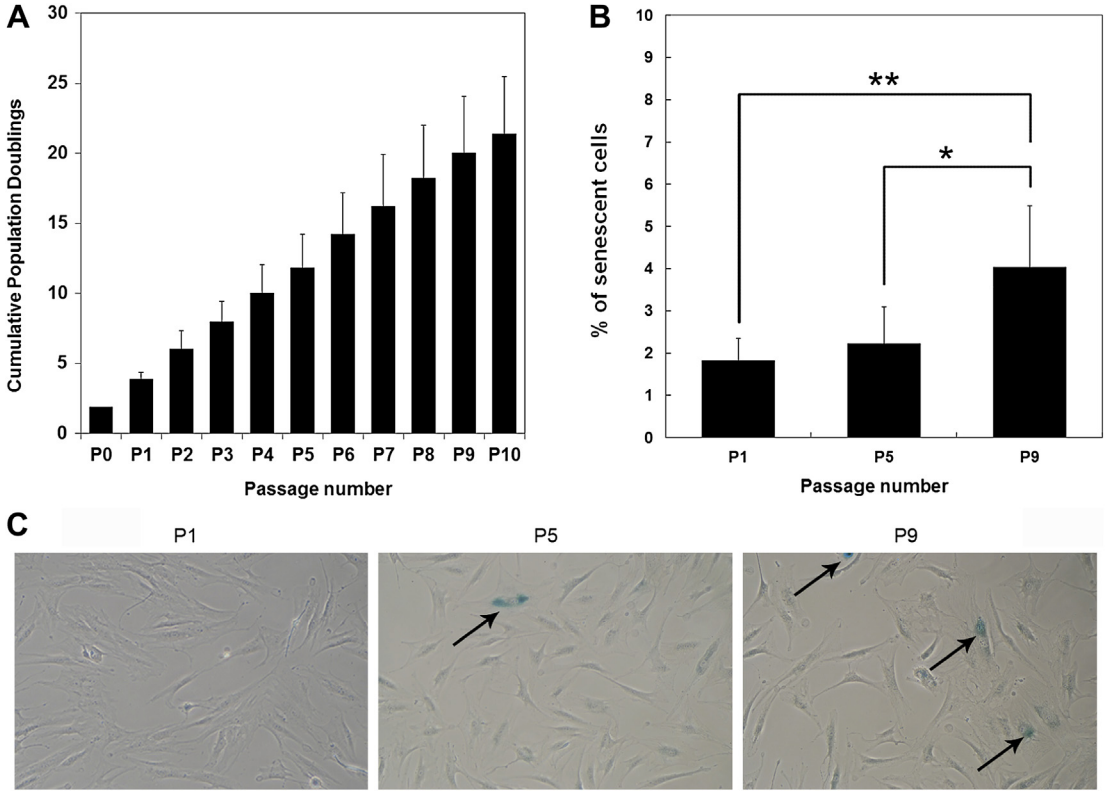


Figure 1 (A) The growth kinetics by cumulative population doublings and (B,C) senescence assay by beta-galactosidase staining of adipose-derived stem cells (ADSCs) ($n = 6$). Arrow: senescent cells (blue). *$p < 0.05$; **$p < 0.01$.

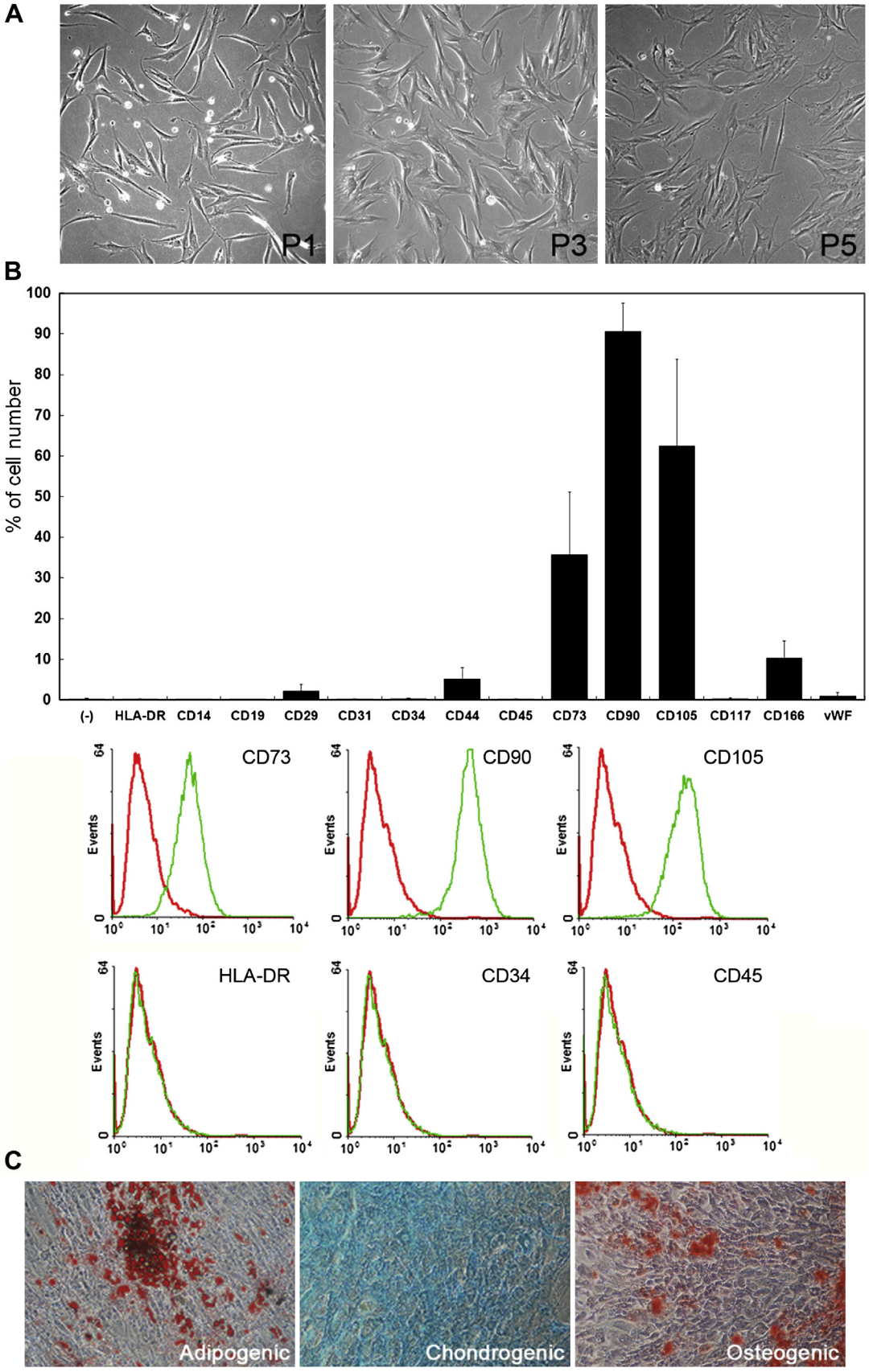
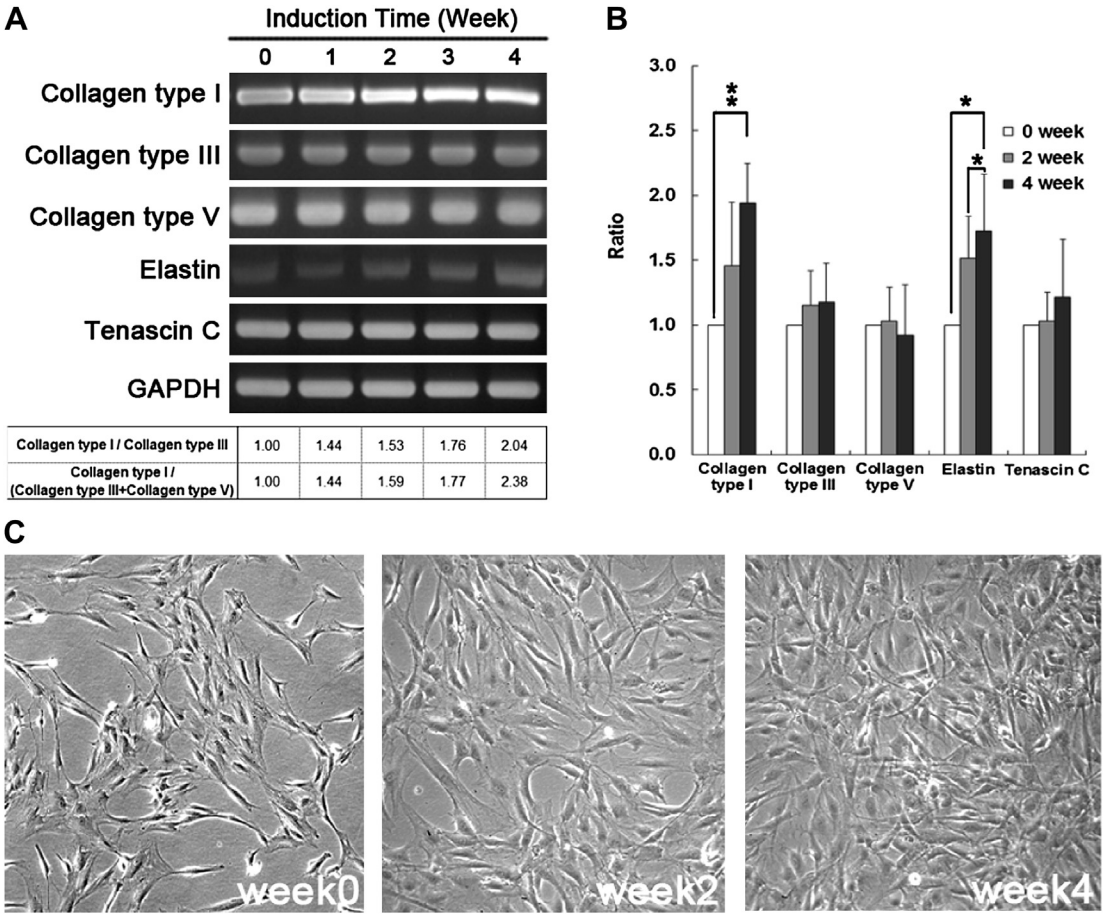


Figure 2 *In vitro* characterization of adipose-derived stem cells (ADSCs) ($n = 6$). (A) Microscopic cell morphology. Original magnification, 100×. (B) Detection of cell surface antigen by flow cytometry. (C) Tri-lineage differentiation potential by cytochemical staining with Oil Red-O (adipogenic), Alcian Blue (chondrogenic), and Alizarin Red S (osteogenic). Original magnification, 100×. P = passage.



Collagen type I / Collagen type III	1.00	1.44	1.53	1.76	2.04
Collagen type I / (Collagen type III+Collagen type V)	1.00	1.44	1.59	1.77	2.38

Figure 3 (A,B) Detection and quantification of the mRNA of extracellular matrix molecules by reverse transcription polymerase chain reaction (RT-PCR), with (C) concomitant monitoring of cell morphological changes during the *in vitro* fibroblastic differentiation of adipose-derived stem cells (ADSCs) ($n = 6$). *$p < 0.05$; **$p < 0.01$.

experimental nude mice is shown. The very loose connective tissue (283.8 ± 44.5 μm) between the superficial and deep muscle layers contributes to a plane where surgical dissection can easily take place for mesh implantation (Fig. 4C–F).

Outcomes of *in vivo* transplantation

The histological findings of the in vivo transplantation of the two tissue-engineered fascia equivalents at 4 weeks and 8 weeks indicated a common trend of mesh degradation, spindle-shaped cell proliferation with matrix deposition, and regression of inflammatory reactions with time. However, the tissue formation from the transplantation of fibroblastic-differentiated ADSCs seemed more fibrotic than that from the transplantation of ADSCs.

At 12 weeks, the subcutaneous transplantation of the tissue-engineered fascia equivalents in nude mice using ADSCs or fibroblastic-differentiated ADSCs could be detected under fluorescence microscopy by the demonstration of DiI-labeled cells (Figs. 5A and 6A). The collagen component was abundant in the neo-tissue formation shown by Masson's Trichrome staining (Figs. 5B and 6B). Furthermore, the neo-tissue formation by the transplantation of ADSCs was a thin (111.0 ± 19.8 μm) and well-organized lamellar structure, which mimicked normal fascia (Fig. 5C,D). By contrast, the neo-tissue formation by the transplantation of fibroblastic-differentiated ADSCs appeared as a thick (414.3 ± 114.9 μm) and undulated, adhesive fibrous tissue, which extended into the surrounding host tissue layers and caused some focal inflammatory reactions (Fig. 6C,D).

The results of the control group of implantation of PLGA mesh mixed with collagen gel without cell seeding were presented in Fig. 7. There was a formation of a thin (102.0 ± 17.1 μm) band of fibrotic tissue and contracture of host tissue at the implantation site. A comparison between the histological outcomes of the normal back skin, the controls, and the treated animals under high-powered magnification is detailed in Fig. 8.

Discussion

In the present study, human ADSCs were prepared and characterized as a group of MSCs. Culturing ADSCs in medium that supports fibroblastic differentiation induced both morphological and gene expression changes in these cells to become functional fibroblast-like cells. Subsequently, we used ADSCs and fibroblastic-differentiated ADSCs to fabricate tissue-engineered fascia equivalents, which were then transplanted subcutaneously in experimental nude mice.

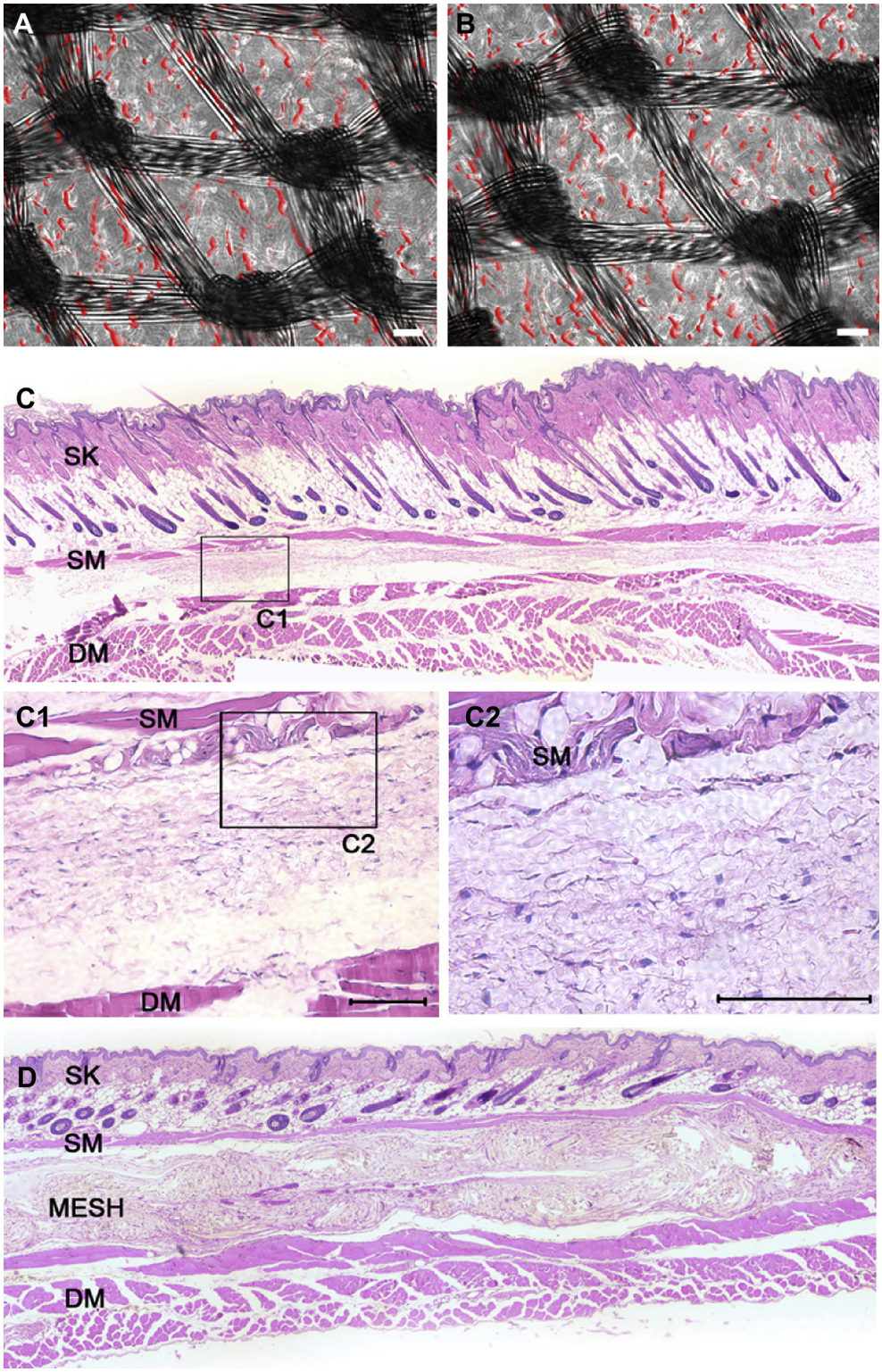


Figure 4 Tissue-engineered fascia equivalents *in vitro* and an experimental animal model. After 24 hours of *in vitro* cultivation, a fascia equivalent was made with biodegradable poly-DL-lactico-glycolic acid (PLGA) mesh and adipose-derived stem cells (ADSCs) (A) or fibroblastic-differentiated ADSCs (B). A very loose connective tissue was noted between the superficial muscle (SM) and deep muscle (DM) layers of the normal back skin in experimental nude mice (C, C1, C2: H&E staining) where mesh was implanted (D). Original magnification, 40× (C,D), 100× (A,B,C1), 200× (C2); internal scale marker = 100 μm. DM = deep muscle; SK = skin; SM = superficial muscle.

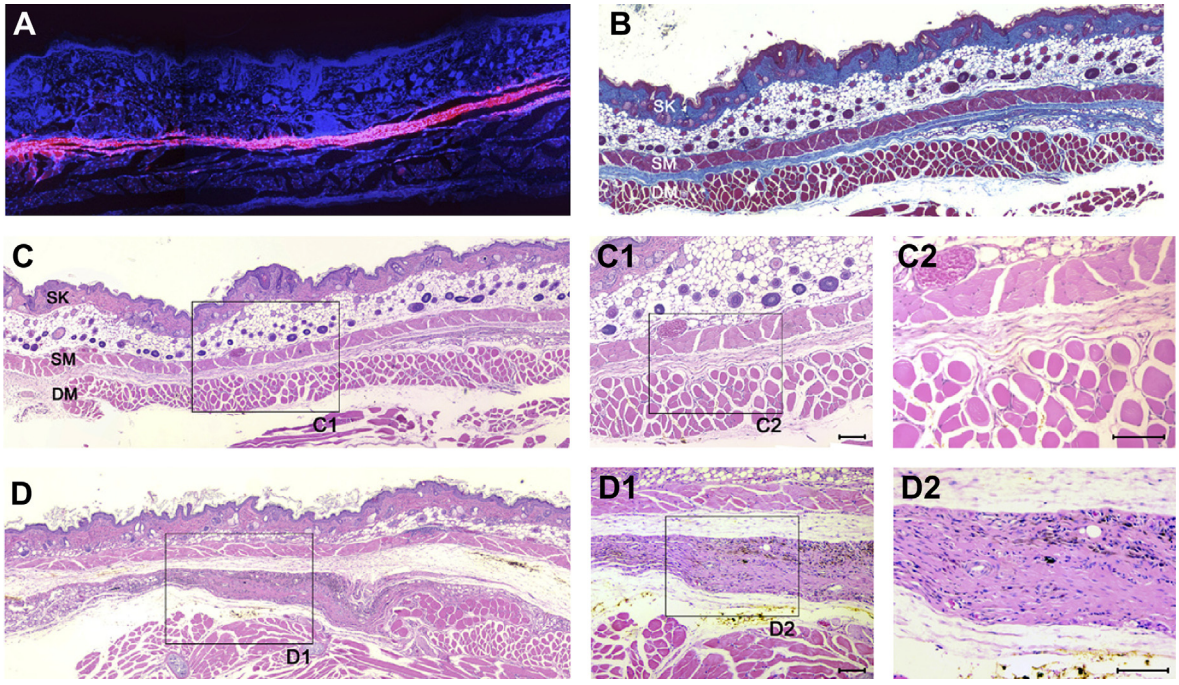


Figure 5 The histological outcomes of the *in vivo* transplantation of the tissue-engineered fascia equivalent using adipose-derived stem cells (ADSCs) after 12 weeks. (A) Under fluorescence microscopy, a thin neo-tissue formation full of the previously DiI-labeled cells (red) is shown. (B) Masson's Trichrome staining revealed collagen content (blue) within the neo-tissue formation between the superficial muscle (SM) and deep muscle (DM) layers. (C,D) H&E staining revealed lamellar, loose connective tissue formation between the SM and DM layers. In specimen (C), fine tissue infiltration into the DM layer (C1—C2) was noted. In specimen (D), graft residual located within the newly formed loose connective tissue was noted (D1—D2). Original magnification, 50× (A,B), 40× (C,D), 100× (C1,D1), 200× (C2,D2); internal scale marker = 100 μm. DM = deep muscle; SK = skin; SM = superficial muscle.

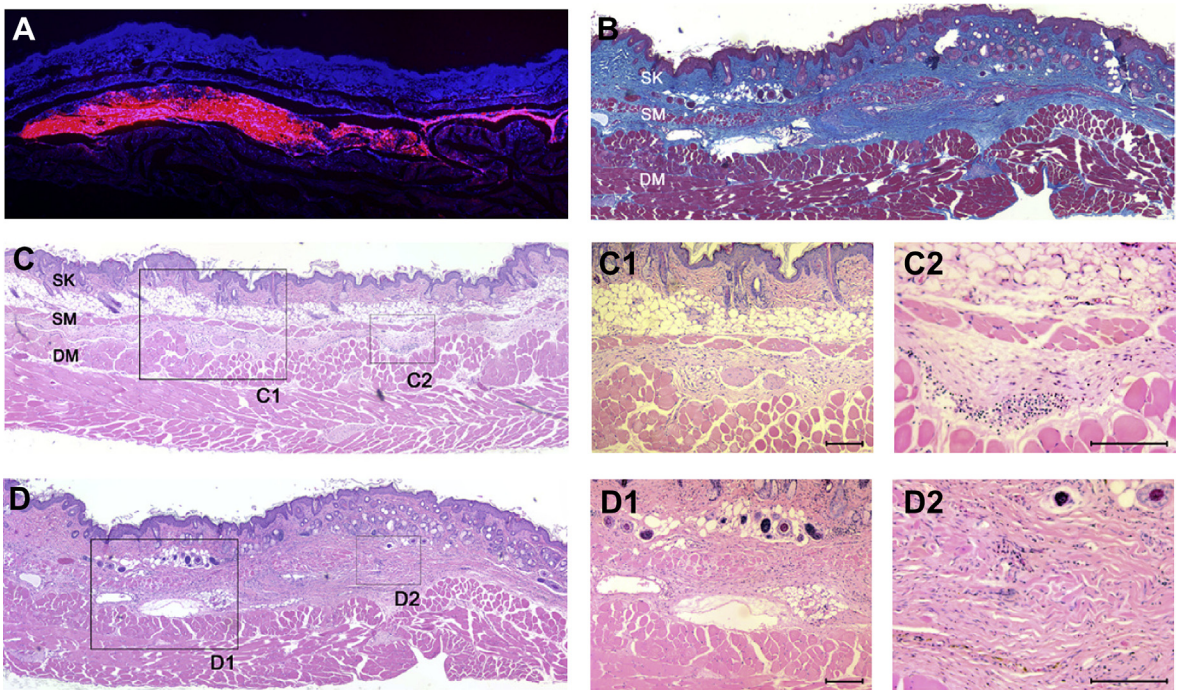


Figure 6 The histological outcomes of the *in vivo* transplantation of the tissue-engineered fascia equivalent using fibroblastic-differentiated adipose-derived stem cells (ADSCs) after 12 weeks. (A) Under fluorescence microscopy, a thick neo-tissue formation full of the previously DiI-labeled cells (red) was noted. (B) Masson's Trichrome staining revealed collagen content (blue) within the neo-tissue formation between the superficial muscle (SM) and deep muscle (DM) layers. (C,D) H&E staining revealed undulated, dense fibrous tissue formation that extends into the SM and DM layers (C1,D2). Focal inflammatory reactions with lymphocytic infiltration (C2) or granulation tissue formation (D1) were noted. Original magnification, 50× (A,B), 40× (C,D), 100× (C1,D1), 200× (C2,D2); internal scale marker = 100 μm. DM = deep muscle; SK = skin; SM = superficial muscle.

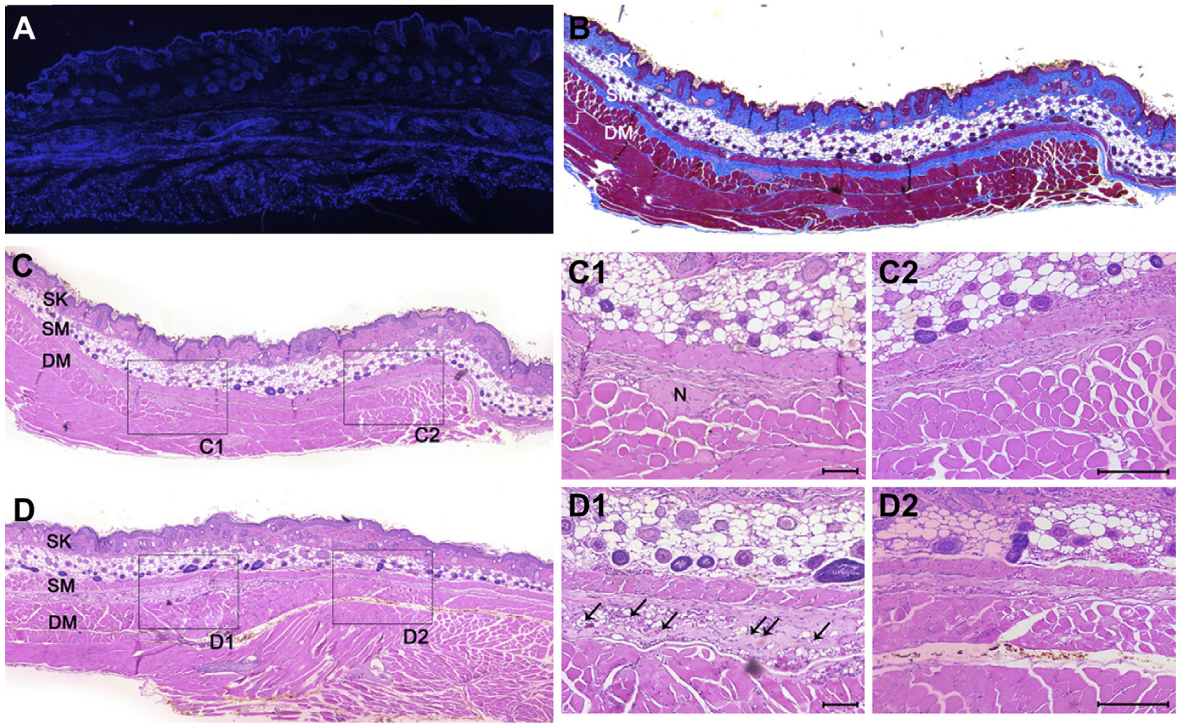


Figure 7 The histological results of the *in vivo* implantation of the poly-DL-lactico-glycolic acid (PLGA) mesh mixed with collagen gel without cell seeding after 12 weeks. (A) No fluorescence signal was detected under fluorescence microscopy. (B) Masson's Trichrome staining revealed collagen content (blue) within the neo-tissue formation between the superficial muscle (SM) and deep muscle (DM) layers. (C,D) A thin band of fibroblast proliferation and dense matrix deposition was noted in the previous implantation site (H&E staining). Within the band, a hypertrophic nerve fiber (C1: N) and areas of matrix nodules admixed with adipocytes (D1: arrows) were seen. Original magnification, 50× (A,B), 40× (C,D), 100× (C1,D1), 200× (C2,D2); internal scale marker = 100 μm. DM = deep muscle; N = nerve; SK = skin; SM = superficial muscle.

Both of the engrafted fascia equivalents could be traced up to 12 weeks after transplantation and resulted in very different histological outcomes, which is a novel finding. Our results suggest that ADSCs could be a potential cell source for tissue-engineered fascia equivalents for the reconstruction of the pelvic floor.

The cell yield [0.8 (0.5—1.1) × 10^5 cells/g] of isolated ADSCs at our laboratory was lower than the results [2.0 (0.5—3.0) × 10^5 cells/g] reported by De Ugarte et al[16] who used similar methodology. The discrepancy in the cell yield may have been confounded by factors such as different donors, sites, and procedures of tissue harvesting.[25,26] Although there was a lower cell yield, ADSCs prepared in our laboratory maintained a relatively constant proliferation rate as well as a low (<5%) level of senescence during extended culture periods. These characteristics are intrinsic properties of stem cells and are essential for implications in cell-based therapies and tissue engineering.[15,16,17,20] Currently, most preclinical and clinical studies on stem cell-based treatment of stress urinary incontinence using bone marrow-derived MSCs or muscle-derived stem cells have shown impressive efficacy.[9,10] However, we suggest that ADSCs might be a better potential cell source than these cells in pelvic floor reconstruction because a large number of stem cells are needed. Human ADSCs have the advantages of being easily harvested in large amounts, cultured, and expanded *in vitro*.[9,15,16,17,20]

Cultured ADSCs in this study were further characterized *in vitro* according to the minimal criteria for defining multipotent MSCs.[21] These cells displayed typical cell morphology, adherence to plastics, and efficient tri-lineage differentiation capacity. Additional cell surface antigens were also detected and quantified by flow cytometry. Our immuno-phenotyping results were consistent with the aforementioned studies[22,23] in that the ADSCs between the third passage to the fifth passage were strongly positive for markers associated with MSCs and negative for those associated with hematopoietic stem cells or endothelial cells. Therefore, the cultured ADSCs prepared in our laboratory for subsequent experiments represent a homogenous population of MSCs. These efforts were made to standardize our cell preparations and allow for comparison between scientific studies among laboratories.

Fibroblastic differentiation of stem cells has widespread significance in the engineering of virtually all tissues including fascia, tendons, ligaments, and as interstitial filler of all organs. Lee et al induced bone marrow-derived MSCs to become mature fibroblast-like cells and found significant increases in the expression of collagen type I and tenascin-C and a loss of multipotent differentiation capacity.[24] We performed fibroblastic differentiation of ADSCs with the aim of engineering fascia for pelvic floor reconstruction to repair POP. In addition to the two ECM markers evaluated by Lee et al, we also evaluated the

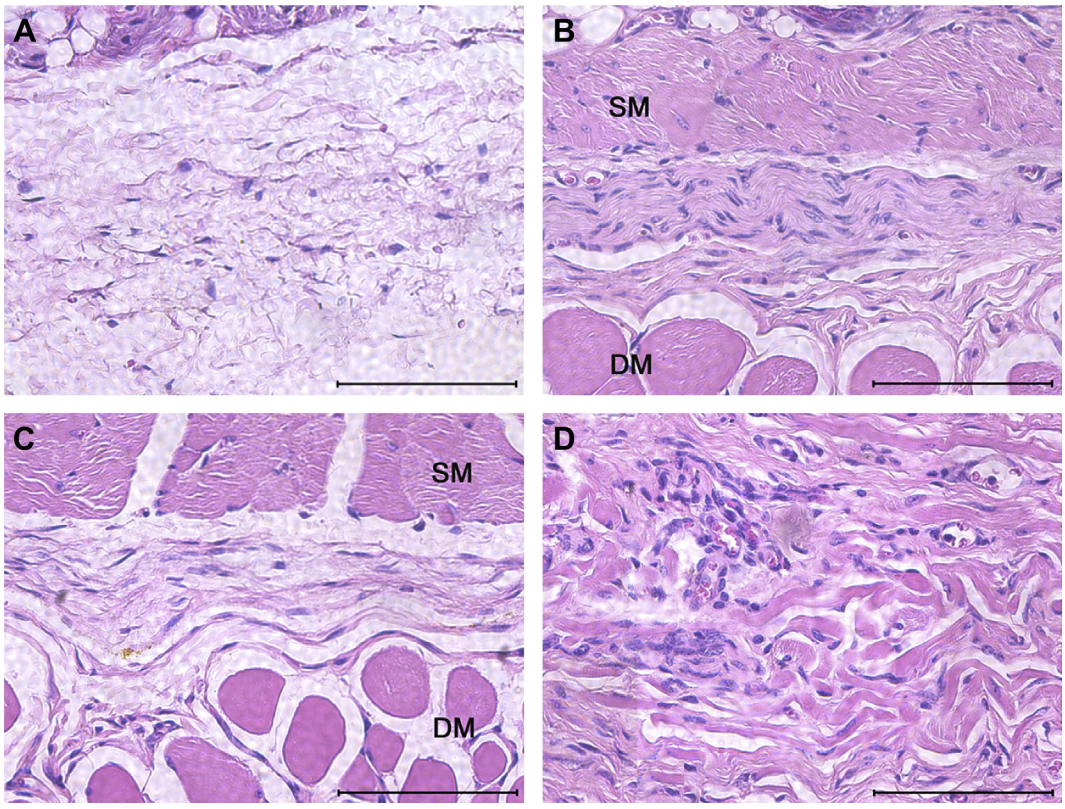


Figure 8 Comparison of the histological characteristics between (A) the normal back skin with loose connective tissue, (B) the control group (scaffold without cell seeding) with a thin but dense band formation, (C) the adipose-derived stem cells (ADSCs) transplantation with lamellar connective tissue formation, and (D) transplantation of fibroblastic-differentiated ADSCs with adhesive fibrotic tissue formation. H&E staining, original magnification, 400× (A—D); internal scale marker = 100 μm. DM = deep muscle; SM = superficial muscle.

mRNA of collagen type III, collagen type V, and elastin via RT-PCR because these molecules are considered important in the vaginal wall and its supportive tissue.[27,28] During the fibroblastic differentiation of ADSCs, we found that there was a statistically significant ($p < 0.05$) increase in gene expression of cellular collagen type I and elastin, and an increase in collagen type I/III and collagen type I/III+V ratios concomitantly with cell morphological changes. These changes may imply that ADSCs after fibroblastic differentiation turned into functional fibroblast-like cells with positively upregulated ECM metabolism in these cells.

The engraftment of tissue-engineered fascia equivalents using ADSCs or fibroblastic-differentiated ADSCs resulted in very different histological outcomes, which imply a potential but different indication of these cells in fascia tissue engineering. Recent studies regarding the vaginal wall and its supportive tissue have demonstrated that the organization of various ECM molecules vary within the different tissues to support normal pelvic function.[27,28,29] Hirata et al[29] conducted a comparative histological study of vaginal supportive tissues according to Delancey's three levels of vaginal support and found that the constitution of collagen, elastin, and smooth muscle fibers varied remarkably at different levels. Based on the histological findings of current studies, we suggest that ADSC transplantation, which results in well-organized lamellar connective tissue formation, might have a potential role in repairing the damaged vaginal fascia in POP patients. Meanwhile, transplantation of fibroblastic-differentiated ADSCs might be developed to suspend (level I) and attach (level II) the vaginal fascia peripherally to the pelvic side walls since dense adhesive fibrous tissue has been formed from transplantation. Furthermore, the implantation of a scaffold without cell seeding (the control group), which resulted in fibrotic tissue deposition and tissue contracture, demonstrated similar findings to current preclinical and clinical studies of surgical grafts implantation.[14,30,31]

The present study has the following limitations: the difficulty of directly translating the knowledge gained from the animal model to humans and the short-term (12 weeks) histological outcomes need to be verified by further long-term studies. Besides, a critical point in this study that needs further clarification is whether the neo-tissue formed directly from transplanted human cells or indirectly by paracrine stimulation of murine resident cells. It has been reported that the therapeutic effects of MSCs might be attributed to several mechanisms including cell differentiation, fusion, paracrine effects and regulation of microenvironment, etc.[32] Although human ADSCs were labeled with a dye (Vybrant DiI; Molecular Probes) before transplantation in this study, the possibility of contamination could not be ruled out. Future work aimed at the distinction of the source of cells and ECM in the neo-tissue formation by advanced cellular and molecular methodologies

may offer insights into the mechanisms through which ADSCs can contribute to fascia repair and regeneration.

In conclusion, our results suggest that an ADSC-seeded implant is better than implant alone in enhancing tissue regeneration after transplantation. ADSCs with or without fibroblastic differentiation might have a potential role in fascia tissue engineering to repair POP in the future. Before this approach can be applied for clinical use, new studies are needed to establish the formation, fate, and function of the tissue-engineered fascia equivalents from ADSCs.

Acknowledgments

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfma.2013.04.017.

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